

Amendments to the Claims:

The listing of claims below will replace all prior versions and listings of claims in the application. The changes to currently amended claims are shown using strikethrough to identify deleted material and underlining to identify added material.

Listing of Claims:

1. (currently amended) A method for detecting a malaria parasite comprising:
releasing the malaria parasite by allowing an erythrocyte in a specimen
treated with an anticoagulant to undergo hemolysis;
staining the released malaria parasite with a fluorescent dye to prepare a
measurement sample;
detecting optical information from the measurement sample; and
distinguishing the malaria parasite based on the optical information obtained;
wherein the specimen is treated with a first hemolytic agent
comprising a first surfactant to form a treated specimen; and
wherein the treated specimen is further treated with a second
hemolytic agent comprising a second surfactant having a hemolytic activity
higher than that of the first surfactant.
2. (original) The method of claim 1, wherein the hemolytic activity of the second
hemolytic agent enables sufficient hemolysis of reticulocyte remaining in the treated
specimen but does not damage the malaria parasite.
3. (original) The method of claim 1, wherein the first surfactant comprises a
nonionic surfactant.
4. (original) The method of claim 1, wherein the second surfactant comprises
an anionic surfactant.
5. (currently amended) A method for detecting a malaria parasite ~~in a sample~~
comprising:

treating ~~the a~~ sample treated with an anticoagulant with a first hemolytic agent comprising a first surfactant to form a first treated sample;

treating the first treated sample with a second hemolytic agent comprising a second surfactant to form a second treated sample, wherein the second surfactant has a hemolytic activity higher than a hemolytic activity of the first surfactant;

staining the second treated sample with a fluorescent dye to form a stained sample;

introducing the stained sample into a flow cytometer;

detecting fluorescent light information; and

distinguishing the malaria parasite based on the fluorescent light information.

6. (original) The method of claim 5, wherein the flow cytometer detects scattering light information.

7. (original) The method of claim 6, wherein the scattering light information comprises scattering light intensity and the fluorescent light information comprises fluorescent light intensity.

8. (original) The method of claim 7, wherein the scattering light intensity and the fluorescent light intensity are used to prepare a two dimensional distribution diagram, wherein a zone corresponding to malaria parasite existence is selected, and wherein a number of cells of the malaria parasite in the zone is counted.

9. (original) The method of claim 5, wherein the fluorescent light information comprises fluorescent light intensity, and wherein the method further comprises:

obtaining a frequency distribution of malaria parasite having a fluorescent light intensity within a range; and

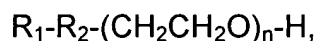
counting a number of cells of the malaria parasite within the range.

10-22. (canceled)

23. (previously presented) The method of claim 5 further comprising detecting scattering light information, wherein the malaria parasite is distinguished based on the fluorescent light information and the scattering light information.

24. (previously presented) The method of claim 1 or 5 wherein the first surfactant and the second surfactant are independently selected from the group consisting of a nonionic surfactant, an anionic surfactant, and a cationic surfactant.

25. (previously presented) The method of claim 24 wherein the nonionic surfactant has a formula:



wherein:

R_1 is an alkyl, alkenyl or alkynyl group having 10 to 22 carbon atoms;

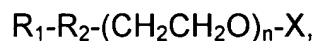
R_2 is -O-, $-(C_6H_4)-O-$ or $-COO-$; and

n is an integer from 8 to 120.

26. (previously presented) The method of claim 25 wherein the nonionic surfactant is a polyoxyethylene (n) alkylphenyl ether.

27. (previously presented) The method of claim 25 wherein the nonionic surfactant is selected from the group consisting of polyoxyethylene (40) nonylphenyl ether, polyoxyethylene (100) nonylphenyl ether, and polyoxyethylene (10) lauryl ether.

28. (previously presented) The method of claim 24 wherein the anionic surfactant has a formula:



wherein:

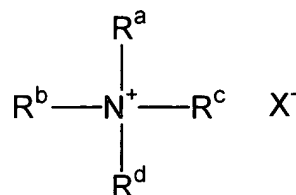
R_1 is an alkyl, alkenyl or alkynyl group having 10 to 22 carbon atoms;

R_2 is -O-, $-(C_6H_4)-O-$ or $-COO-$;

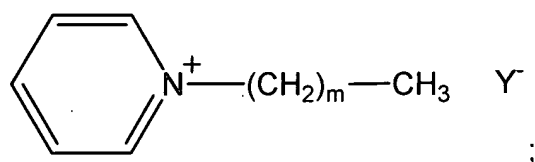
n is an integer from 8 to 120; and

X is SO₃Na, COONa, OSO₃Na or ONa.

29. (previously presented) The method of claim 24 wherein the cationic surfactant has a formula selected from the group consisting of



and



wherein:

R^a is an alkyl, alkenyl or alkynyl group having 8 to 20 carbon atoms;

each of R^b, R^c and R^d, independently, is an alkyl, alkenyl or alkynyl group having 1 to 8 carbon atoms;

each of X⁻ and Y⁻, independently, is an anion; and

m is an integer from 7 to 19.

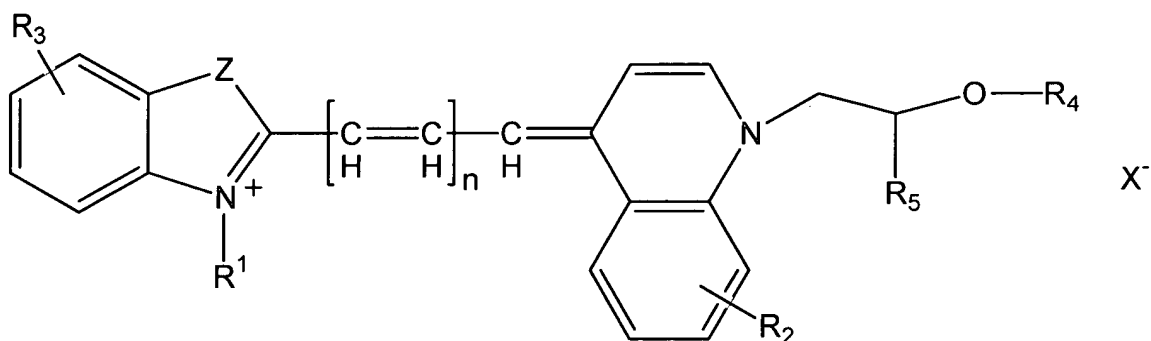
30. (previously presented) The method of claim 29 wherein the cationic surfactant is selected from the group consisting of octyltrimethylammonium bromide, decyltrimethylammonium bromide, lauryltrimethylammonium chloride, myristyltrimethylammonium bromide, and cetylpyridinium chloride.

31. (previously presented) The method of claim 1 or 5 wherein the first surfactant and the second surfactant are independently selected from the group consisting of polyethylene glycol mono[p-(1,1,3,3-tetramethylbutyl)phenyl] ether, 3-[(3-chloroamidopropyl)dimethylammonio] propanesulfonic acid, 3-[(3-chloroamidopropyl)dimethylammonio]-2-hydroxypropanesulfonic acid, N,N-bis(3-D-gluconamidopropyl)deoxychloramide, sucrose monocaprate, sucrose monocholate, n-octyl-α-D-glucopyranoside, n-heptyl-α-D-thioglucopyranoside, n-octyl-α-D-

thioglucopyranoside, n-dodecyl- α -D-maltopyranoside, and n-nonyl- α -D-thiomaltopyranoside.

32. (previously presented) The method of claim 1 or 5 wherein the fluorescent dye is selected from the group consisting of acridine orange, thiazole orange, ethidium bromide, propidium iodide, auramine O, Hoechst 33258, Hoechst 33342, rhodamine 123, and DiOC1(3).

33. (previously presented) The method of claim 1 or 5 wherein the fluorescent dye has a structure:



wherein:

R_1 is a hydrogen atom or a lower alkyl group;

each of R_2 and R_3 , independently, is a hydrogen atom, a lower alkyl group or a lower alkoxy group;

R_4 is a hydrogen atom, an acyl group or a lower alkyl group;

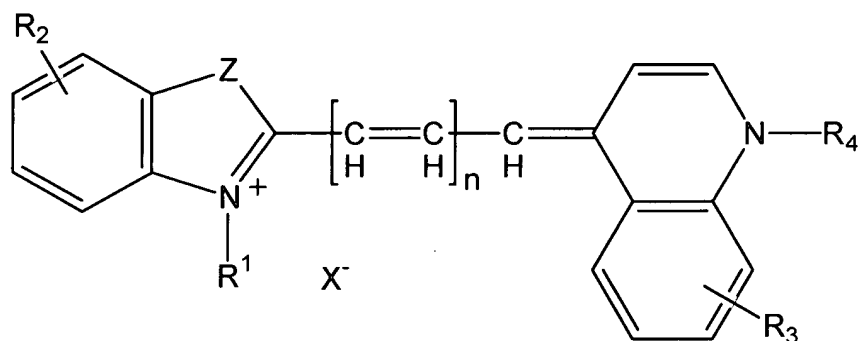
R_5 is a hydrogen atom or an optionally substituted lower alkyl group;

Z is a sulfur atom, an oxygen atom or a carbon atom substituted by two lower alkyl groups;

n is 1 or 2; and

X^- is an anion.

34. (previously presented) The method of claim 1 or 5 wherein the fluorescent dye has a structure:



wherein:

R_1 is a hydrogen atom or an alkyl group;

each of R_2 and R_3 , independently, is a hydrogen atom, a lower alkyl group or a lower alkoxy group;

R_4 is a hydrogen atom, an acyl group or an alkyl group;

Z is a sulfur atom, oxygen atom or carbon atom substituted by two lower alkyl groups;

n is 0, 1 or 2; and

X^- is an anion.

35. (previously presented) The method of claim 1 or 5 wherein the fluorescent dye is selected from the group consisting of acridine homodimer, actinomycin D, 7-aminoactinomycin D, 9-amino-6-chloro-2-methoxyacridine, BOBO-1, BOBO-3, BO-PRO-1, BO-PRO-3, 4',6-diamidino-2-phenylindole, dihydroethidium, 4',6-(diimidazolin-2-yl)-2-phenylindole, ethidium-acridine heterodimer, ethidium diazide, ethidium homodimer-1, ethidium homodimer-2, ethidium monoazide, hexidium iodide, hydroxystilbamidine, methanesulfonate, LDS 751, OilGreen ssDNA quantitation reagent, PicoGreen dsDNA quantitation reagent, PO-PO-1, PO-PO-3, PO-PRO-1, PO-PRO-3, SYTO 11 live-cell nucleic acid stain, SYTO 12 live-cell nucleic acid stain, SYTO 13 live-cell nucleic acid stain, SYTO 14 live-cell nucleic acid stain, SYTO 15 live-cell nucleic acid stain, SYTO 16 live-cell nucleic acid stain, SYTO 20 live-cell nucleic acid stain, SYTO 21 live-cell nucleic acid stain, SYTO 22 live-cell nucleic acid stain, SYTO 23 live-cell nucleic acid stain, SYTO 24 live-cell nucleic acid stain, SYTO 25 live-cell nucleic acid stain, SYTO 17 red fluorescent nucleic acid stain, SYTOX green nucleic

acid stain, TO-PO-1, TO-PRO-3, TO-PRO-5, TO-TO-1, TO-TO-3, YO-PRO-1, YO-PRO-3, YO-YO-1, and YO-YO-3.

36. (new) The method of claim 1 wherein the anticoagulant is selected from the group consisting of EDTA, heparin, citric acid, citrate, and combinations thereof.

37. (new) The method of claim 5 wherein the anticoagulant is selected from the group consisting of EDTA, heparin, citric acid, citrate, and combinations thereof.

SUPPORT FOR AMENDMENTS

The amendments to independent claims 1 and 5 are fully supported by the description in the specification (e.g., page 10, line 25 to page 11, line 2).

New dependent claims 36 and 37 are supported by the description in the specification (e.g., page 11, lines 2-4).

No new matter has been added. Upon entry of this Response, claims 1-9 and 23-37 are present and active in the application.